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Article *in* Trends in Biochemical Sciences · February 2003

DOI: 10.1016/S0968-0004(02)00007-5 · Source: PubMed

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Historical review: Viruses, crystals and geodesic domes

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In the mid 1950s, Francis Crick and James Watson attempted to explain the structure of spherical viruses. They hypothesized that spherical viruses consist of 60 identical equivalently situated subunits. Such an arrangement has icosahedral symmetry. Subsequent biophysical and electron micrographic data suggested that many viruses had >60 subunits. Drawing inspiration from architecture, Donald Caspar and Aaron Klug discovered a solution to the problem – they proposed that spherical viruses were structured like miniature geodesic domes.

June 2002 marked the 40th anniversary of the public presentation of the Caspar–Klug theory of virus structure at the Cold Spring Harbor Symposium 'Basic Mechanisms in Animal Virus Biology' [1]. Inspired by Buckminster Fuller's geodesic domes, Donald Caspar and Aaron Klug proposed that 'spherical' viruses were made from identical subunits bonded together in 'quasi-equivalent' ways. The specific contact pattern among adjacent viral subunits is conserved, but there can be slight deformation of some bonds from their optimal angles and lengths. They also derived a remarkably simple selection rule that describes the number of structural subunits (S) that a spherical virus can have (Eqn 1) where f is a positive integer, h and k are integers with no common factor, and T is known as the triangulation number or T-number of the virus.

$$S = 60T = 60(h^2 + hk + k^2)f^2 \quad \text{(Eqn 1)}$$

The Caspar–Klug theory provided an elegant solution to the problem of how to construct spherical viruses from >60 identical protein molecules. The central ideas of the Caspar–Klug theory were taken to be true of all spherical viruses until the early 1980s, when it was discovered that the polyoma virus capsid, an apparent $T = 7$ structure, actually has 360 subunits – a number disallowed by the selection rule [2]. Nonetheless, the majority of known spherical viruses obey the Caspar–Klug selection rule and T-numbers are still widely used to classify viral shells.

Virus crystallography in the mid 1950s

Originally trained as physicists, both Caspar and Klug came to study spherical viruses via earlier investigations into the structure of rod-shaped tobacco mosaic virus (TMV). In 1953, after studying physics at Cornell University (Ithaca, NY, USA), Donald Caspar began his PhD in biophysics at Yale University (New Haven, CT, USA). His goal was to pass X-rays through an oriented fibre of TMV particles and then analyze the diffracted X-rays to infer how the virus is structured. In the same year, Klug finished his PhD in solid-state physics at the University of Cambridge (UK) and began a Nuffield post-doctoral fellowship with Harry Carlisle at Birkbeck College (London, UK) on a relatively uninspiring ribonuclease structure project. At the beginning of 1954, Klug fortuitously met Rosalind Franklin on the stairs of the Torrington Square house-turned-laboratory while she was carrying some of her diffraction photographs. After seeing her fascinating X-ray diffraction patterns of TMV, he changed the focus of his research to virus crystallography, thereby beginning arguably Franklin's most important scientific collaboration. James Watson also made a foray into TMV research; he was interested in RNA and hoped that the study of TMV would enable him to solve the RNA structure (TMV contains RNA as its genetic material). In 1954, with help from Francis Crick, Watson published a paper showing that TMV was helical but, as it turned out, he was unable to determine the correct number of subunits per turn and was later corrected by Franklin [3].

Unlike TMV, which forms fibres, spherical viruses form true crystals. John Desmond Bernal and his colleagues (Birkbeck College) had taken powder diffraction photographs of tomato bushy stunt virus (BSV) in 1938 but, partially because of the Second World War, it was not until the late 1940s that crystallographers began single crystal studies. Crick developed an enduring interest in viruses during his war-time service in the Admiralty. During the 1954 Cold Spring Harbor phage meeting, Caspar spoke with Crick and Watson about Dorothy Crowfoot Hodgkin's hypothesis that spherical viruses have cubic symmetry. In addition, Crick and Watson thought spherical viruses probably had icosahedral symmetry. Cubic symmetry involves having at least four threefold rotational axes. For example, all of the platonic solids – the tetrahedron, the cube, the octahedron, the dodecahedron and the icosahedron – have cubic symmetry. Imagine a cube; if you look down the body-diagonal, you are looking down a threefold rotational axis. A cube has four such axes, as do all the platonic solids. In December of 1954, Caspar traveled to Caltech (CA, USA) where Watson was working. Caspar planned to analyze southern bean mosaic virus, a spherical virus, but unfortunately, Caltech did not have a sufficiently powerful or adjustable X-ray source. To make

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further progress he would have to go to England, where there were powerful X-ray tubes.

In the interim, Caspar analyzed his TMV data. He calculated a cylindrically averaged radial mass distribution function for TMV. Watson and Caspar speculated about the nature of TMV RNA given Caspar's results. They proposed a 10–12 stranded RNA helix surrounded by TMV protein, a structure that they considered very pretty, but speculative. However, they were forced to abandon their pretty model when they saw Franklin's data. Consequently, they never published their interpretation. In London, Franklin was also analyzing TMV structure – she calculated a radial distribution function for repolymerized TMV protein without the RNA. Using Franklin's data (from virus protein) in conjunction with Caspar's (from virus protein and RNA), one could determine the location of RNA in TMV [4,5]. Surprisingly, it was not in the core of the virus, as might be suspected if the TMV protein formed a 'container' for the fragile RNA, nor in the inner most peak of density, but 40 Å from the center.

The Crick–Watson theory of virus structure

At the end of the summer of 1955, Caspar traveled to England, where he met Franklin and Klug. In Cambridge, Caspar began to analyze BSV and, a little later in London, Klug began to analyze turnip yellow mosaic virus (TYMV), another well-studied spherical virus. Results came quickly for Caspar, but they were unexpected and novel. He discovered 'spikes' in the BSV diffraction pattern that indicated fivefold symmetry [6]. Max Perutz coined the term 'spike' while helping Caspar refine his manuscript. Fivefold symmetry indicated that the virus possessed icosahedral (or 532) symmetry (icosahedra and dodecahedra possess fivefold symmetry in addition to cubic symmetry). Of the platonic solids, only the icosahedron (20 triangles) and the dodecahedron (12 pentagons) have 532 symmetry. Caspar informed Crick and Watson that he had experimental evidence for their hypothesis that spherical viruses have icosahedral symmetry. With the knowledge of Caspar's results, which confirmed their earlier theoretical speculation, Crick and Watson re-wrote their article and submitted it to *Nature* [7]. They argued that 'a virus possessing cubic symmetry must necessarily be built from a regular aggregation of smaller asymmetrical building bricks, and this can only be done a very limited number of ways'. These ways correspond to the three cubic point-groups. A point-group is a mathematical entity consisting of symmetry elements whose axes and/or planes of symmetry intersect at one point. A virus with the symmetry of a cubic point-group consists of a maximum of 12, 24 or 60 identically situated 'subunits' corresponding to tetrahedral, octahedral or icosahedral symmetry, respectively. Further division of these asymmetrical subunits would lead to differently situated 'sub-subunits' and would destroy the repeated identical packing.

Caspar published his experimental results for BSV immediately following Crick and Watson's article in *Nature* [6]. In March of 1956, Crick presented the Crick–Watson theory and Caspar's experimental results to a group of virologists at a small Ciba conference in London [8] (Fig. 1). He argued that given the size of a small viral genome and a coding ratio of 3:1, there is not enough information in the viral genome to encode a large number of non-identical protein subunits. Therefore, Crick concluded that there must be one subunit that is repeated numerous times. His ideas were met with some skepticism among the traditional animal virologists, who were not used to thinking in terms of genetic information. In the discussion session following the paper, Caspar illustrated several ideas with ping-pong ball models that he had built to illustrate the icosahedral symmetry of BSV. Klug mentioned that he had obtained similar results for TYMV.

A week later, at the International Union of Crystallography meeting in Madrid (Spain), Caspar presented a paper that combined Crick and Watson's theoretical speculations and his experimental results (Fig. 2). The paper, entitled 'The molecular viruses considered as point-group crystals', illustrated how Crick, Watson and Caspar were thinking of a virus particle as a type of crystal. They conceived of the virus as made up of identical subunits bonded together in identical ways, with each subunit equivalent to every other subunit, as is true of crystals. But the idea was that viral proteins, in contrast to potentially unbounded space-group crystals, form bounded containers or 'surface crystals' with point or line symmetry. The maximum number of equivalently related subunits in a cubic point-group crystal is 60 – imagine each of the 20 triangular sides of an icosahedron divided into three. Crystallographers were reluctant to believe that Caspar had discovered fivefold symmetry. As

Fig. 1. From left to right: Michael Stoker, James Watson, Milton Salton, and Francis Crick at the 1956 Ciba Foundation symposium, 'The Nature of Viruses'. ©The Novartis Foundation (www.novartisfound.org.uk), formerly the Ciba Foundation. Reproduced, with permission.

Fig. 2. From left to right: Anne Cullis, Francis Crick, Donald Caspar, Aaron Klug, Rosalind Franklin, Odile Crick and John Kendrew at the 1956 International Union of Crystallography meeting in Madrid, Spain. Image courtesy of Donald Caspar.

any student of crystallography knows, true fivefold symmetry of a crystal lattice is impossible. Caspar was not proposing an impossibility but, rather, that the virus itself, not the crystal lattice, contained fivefold symmetry. It is possible to crystallize a particle possessing fivefold symmetry in a lower symmetry lattice. Nonetheless, fivefold-symmetrical macromolecules are rare and his audience remained suspicious. Klug also presented a paper at the Madrid conference; his paper concerned the Fourier transforms of 23, 432 and 532 point-groups expressed in terms of linear combinations of spherical harmonics and Bessel functions. However, even though Caspar and Klug were pursuing complementary research programs, their collaboration would not begin for another two years.

Caspar and Klug's collaboration

Caspar and Klug's collaboration began during great sorrow. In August 1958, Franklin was scheduled to talk in Bloomington (IN, USA) at a plant pathology meeting organized to celebrate the 50th anniversary of the American Phytopathology Society. She would have spoken on her recent work on TMV. Tragically, Franklin died of ovarian cancer in April 1958 at the age of 37. Under these unfortunate circumstances, Caspar was invited by the committee to speak in Franklin's place, and he suggested Klug's co-authorship. They wrote a review of the X-ray diffraction of viruses focusing particularly on Franklin's most recent TMV work, and she was listed posthumously as first author [9].

Klug and his colleagues, Kenneth Holmes and John Finch, expanded their research program to include polio as well as TYMV. One of Buckminster Fuller's British popularizers [10], John McHale, read of Klug and Finch's work on Polio in *The Observer* (June 21, 1959) and noted the similarities between viral structure and Fuller's geodesic domes. It is not altogether surprising that it

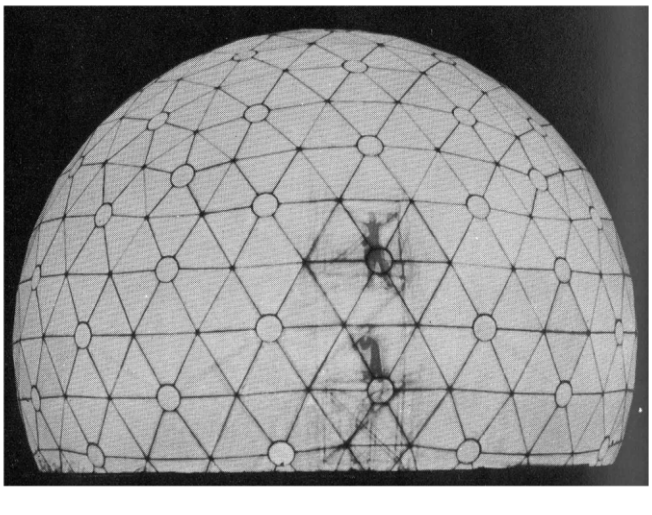

Fig. 3. Geodesic dome, often used to house radar equipment. Note the quasi-equivalent triangular subunits arranged into groups of five and six.

was McHale who saw a connection between domes and viruses. He was a member of the 'Independent Group', a loose-knit group of rebellious young artists and art critics based in London, who promoted a new conception of art that focused on the aesthetic value of technology and popular culture [11]. They organized exhibitions such as 'Growth and Form' and 'Parallel of Life and Art' in their search for new sources of aesthetic value. McHale corresponded with Klug to arrange a meeting between Buckminster Fuller and Klug in London, in July 1959. However, the first meeting between Fuller and Klug yielded no significant insights into viral structure beyond the obvious similarity that both domes and viruses have icosahedral symmetry.

New evidence that many viruses had more complicated structures than could be accounted for by Crick and Watson's model came from a different direction. The electron microscopy of viruses was significantly improved by the new technique of 'negative staining', popularized by the microscopist Robert Horne and Sydney Brenner, both of whom also worked in Cambridge [12]. In 1959, Horne and the virologist Peter Wildy used the technique to examine a variety of viruses at a resolution high enough to observe that negatively stained viruses had a more complicated sub-structure than could be explained with a 60-subunit model [13]. Existing biophysical data supported this result. A new theoretical idea was needed because it is physically impossible to enclose space with >60 equivalently placed identical subunits. After reflecting upon the pictures of domes in Robert Marks' book, *The Dymaxion World of Buckminster Fuller*, Caspar and Klug, somewhat independently, hit upon the idea of 'near-equivalence', which Klug aptly coined 'quasi-equivalence' [14]. The idea was that identical viral subunits could bind together in quasi-equivalent positions to form a shell with >60 subunits while conserving the same specific contact pattern between subunits. Consider the triangular 'subunits' of geodesic domes. The subunits lie in (at least) two quasi-equivalent positions, for example, those in pentamers (rings of five) and those in hexamers (rings of six)

(Fig. 3). The relative angles between the neighbors differ slightly for the two types of subunit positions, but the types of physical contact are conserved. Quasi-equivalent bonding loosens the constraints on the number of subunits in the structure. In the point-group crystal viruses, the maximum number of identically situated subunits was 60. With quasi-equivalence, much higher numbers are possible. However, even with quasi-equivalence, not just any number of subunits is allowed – there are still stringent constraints upon the number of subunits a virus can have.

Caspar and Klug's famous paper of 1962

Back in the USA, while considering hexagonal nets, Caspar derived a simple selection rule that described all the possible icosahedral quasi-equivalent structures (Eqn 1). In the meantime, Klug was invited to speak at the 1962 Cold Spring Harbor meeting (NY, USA). The invitation sparked a further collaborative paper. The transatlantic collaboration was extremely fruitful and Casper and Klug soon had too much material for one paper. They literally cut up Caspar's original notes and reassembled them with new material. The first paper [1] would consider the geometrical aspects of virus design and assembly, and the second, physical aspects of viral design and assembly. The second paper, although promised in the first, was never published, although the crucial ideas and illustrations finally appeared in a belated article, entitled 'Quasi-equivalence revisited', published by Caspar in 1980 [15]. In their 1962 Cold Spring Harbor paper, Caspar and Klug coined several new terms. They proposed that icosahedral virus shells can be classified according to their T-number. This number represents one-third of the number of protein subunits on each face of the icosahedral virus particle; thus, 60T is the number of subunits in a complete 20-sided capsid. Because only certain T-numbers are possible, the Caspar–Klug theory predicts what can and cannot occur in nature. They also introduced the term 'self-assembly', which describes how structural proteins, under the right conditions, aggregate correctly into virus shells and other macromolecular assemblies without any external aid. This new term was introduced with an extension to the crystal analogy:

'Self assembly is a process akin to crystallization and is governed by the laws of statistical mechanics. The protein subunits and the nucleic acid chain spontaneously come together to form a simple virus particle because this is their lowest energy state' [1].

The 1956 crystal metaphor of Caspar, Crick and Watson was static. In 1962, Caspar and Klug utilized the dynamic nature of crystal growth. This reflects a general trend in their research; as time progressed, Caspar and Klug considered virus production from a more dynamic perspective. The long-term goal was a complete explanation of how viruses assembled, in addition to a description of the final assembled product.

One influential concept, not explicitly mentioned in the 1962 paper but discussed in the promised unpublished paper, is the notion of 'tensegrity' [15]. The basic idea of a tensegrity structure or sculpture is to isolate the components of the structure that are under tension (wires) and those under compression (struts). Much like a simple tent, the rigid struts or poles are under compression and do not touch, whereas the wires remain under tension. Although there is some controversy over the origins of the concept, the American sculptor Kenneth Snelson constructed the first tensegrity structure and/or sculpture in 1948 while he was a student of Buckminster Fuller at Black Mountain College (NC, USA). Perhaps his most famous sculpture is 'Needle Tower', exhibited at the Hirshhorn Sculpture Garden on the Mall in Washington, DC (USA) [16]. The struts of icosahedrally symmetrical tensegrity structures naturally arrange into quasi-equivalent positions. Tensegrity structures are in equilibrium and will return to their original state after moderate deformation. In an analogous way, based on thermodynamic considerations, Caspar and Klug proposed that the structures of viruses are in their lowest energy state.

Fig. 4. Donald Caspar with his D-stix models of icosageodesic surface lattices and his T = 3 peg model at Buckminster Fuller's Boston residence in February 1962. At the time, Fuller was the Charles Elliot Norton Visiting Professor of Poetry at Harvard. Fuller's grandson is partially pictured on the right. Image courtesy of Donald Caspar.

Summary

To recapitulate, the biophysicists Caspar and Klug began their respective virology careers by working on the rod-shaped TMV. For Caspar, his TMV work brought him into professional contact with Watson. Crick and Watson argued, using theoretical principles, that small spherical viruses, such as BSV and TYMV, were constructed out of identical subunits in identical environments. They thought of viruses as 'point-group crystals'. This analogy is perhaps not too surprising, as Crick and Watson practiced crystallography and were keen model-builders. The maximum number of subunits that can be arranged in a cubic 'point-group crystal' virus is 60. However, evidence from electron microscopy and biophysical techniques suggested that most spherical viruses had >60 protein subunits. After reading Robert Marks' book on Buckminster Fuller, Caspar and Klug developed the idea that virus shells were structured like geodesic domes (Fig. 4). Subunits on many geodesic domes are not equivalently related but, rather, quasi-equivalently related. If viral subunits are quasi-equivalently related, then there can be

>60 subunits per virus. They also developed the idea of 'self assembly' after considering the viral assembly process as a type of crystallization process. In an unpublished paper, Caspar and Klug pointed out the analogy between viruses and tensegrity structures. Both the crystallization and the tensegrity analogies legitimate further work on the thermodynamics of viral assembly in which viral shells are taken to be minimum energy structures. The early history of structural virology can be seen as a succession of macroscopic analogies applied to the microscopic realm.

Acknowledgements

I thank the many people who kindly agreed to share their knowledge of the period, including Sydney Brenner, Carolyn Cohen, Francis Crick, John Finch, Alfred Gierer, Ken Holmes, Robert Horne, Hugh Huxley, Vittorio Luzzati, Max Perutz, Alex Rich, James Watson, Jo Wildy and especially Don Caspar and Aaron Klug. My colleagues Peter Achinstein, Angela Creager, Lindley Darden, Ed Lattman, and Bob Olby provided useful comments. The Novartis Foundation (formerly Ciba) allowed me access to their archives, as did Stanford University, California Institute of Technology, the University of Maryland at Baltimore County, The Tate Gallery and Churchill College, Cambridge. Jeremy Norman generously allowed me access to what must be the most impressive private archive for the history of molecular biology. This work was supported by NSF Doctoral Dissertation Award 9910891.

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